Cell Reports

Supplemental Information

A Role for IFITM Proteins in Restriction of *Mycobacterium tuberculosis* Infection

Shahin Ranjbar, Viraga Haridas, Luke D. Jasenosky, James V. Falvo, and Anne E. Goldfeld

Figure S1

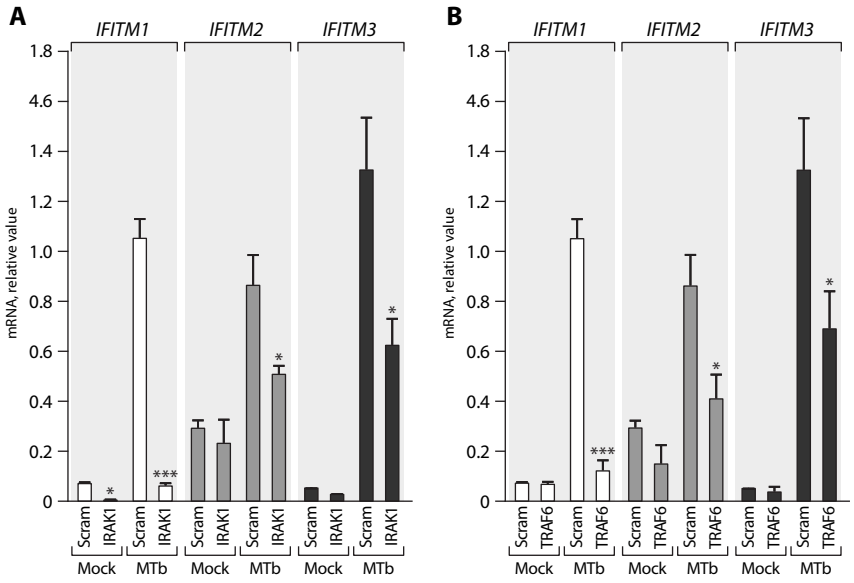

A
IFITM1
IFITM2
IFITM3
mRNA, relative value
1.8
4.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
Scram
IRAK1
Mock
MTb
B
TRAF6

Figure S2

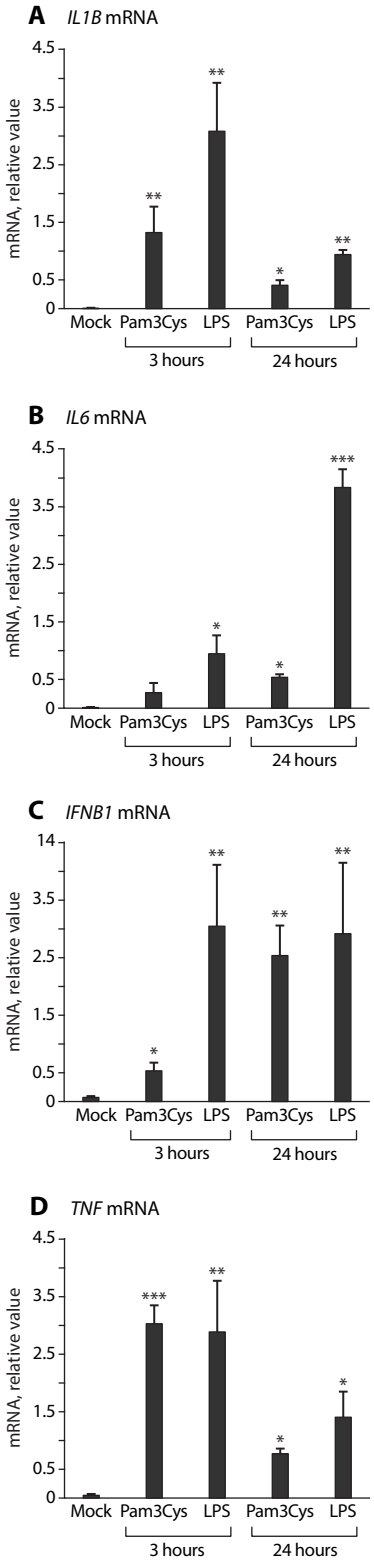

A IL1B mRNA
B IL6 mRNA
C IFNB1 mRNA
D TNF mRNA
mRNA, relative value
Mock
Pam3Cys
LPS
Pam3Cys
LPS
3 hours
24 hours

Figure S3

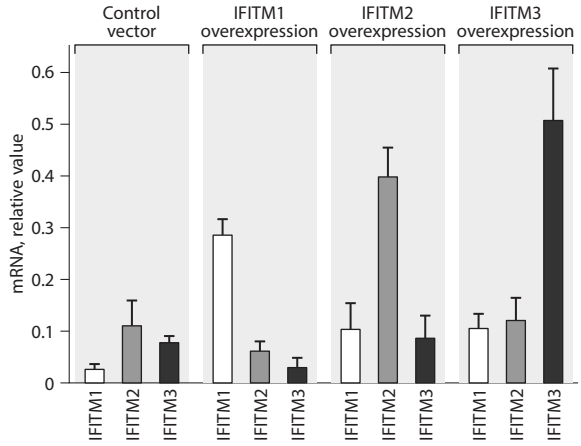

Control vector
IFITM1 overexpression
IFITM2 overexpression
IFITM3 overexpression
mRNA, relative value
0
0.1
0.2
0.3
0.4
0.5
0.6
IFITM1
IFITM2
IFITM3

Figure S4

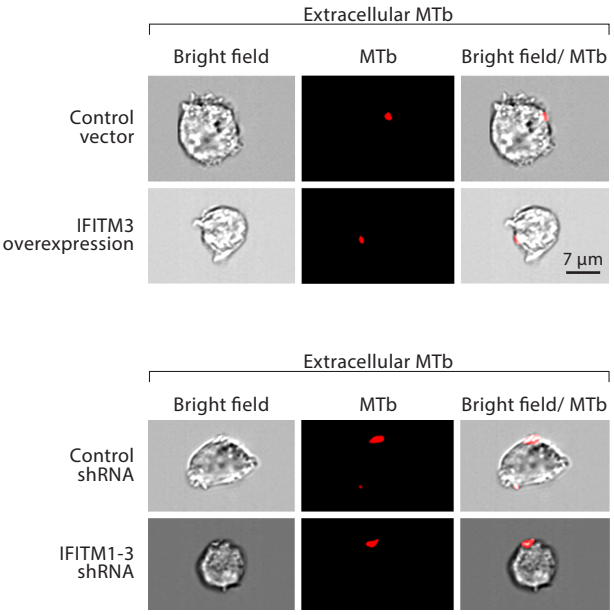

Extracellular MTb
Bright field
MTb
Bright field/ MTb
Control vector
IFITM3 overexpression
7 µm
Extracellular MTb
Bright field
MTb
Bright field/ MTb
Control shRNA
IFITM1-3 shRNA

Figure S5

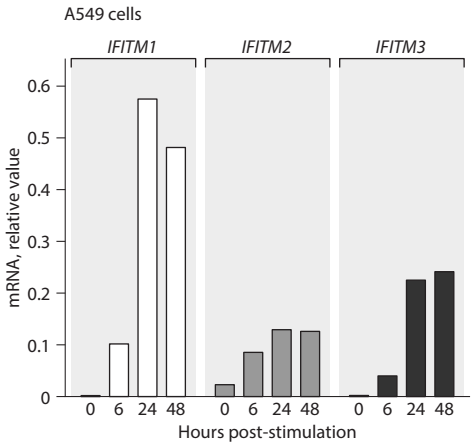
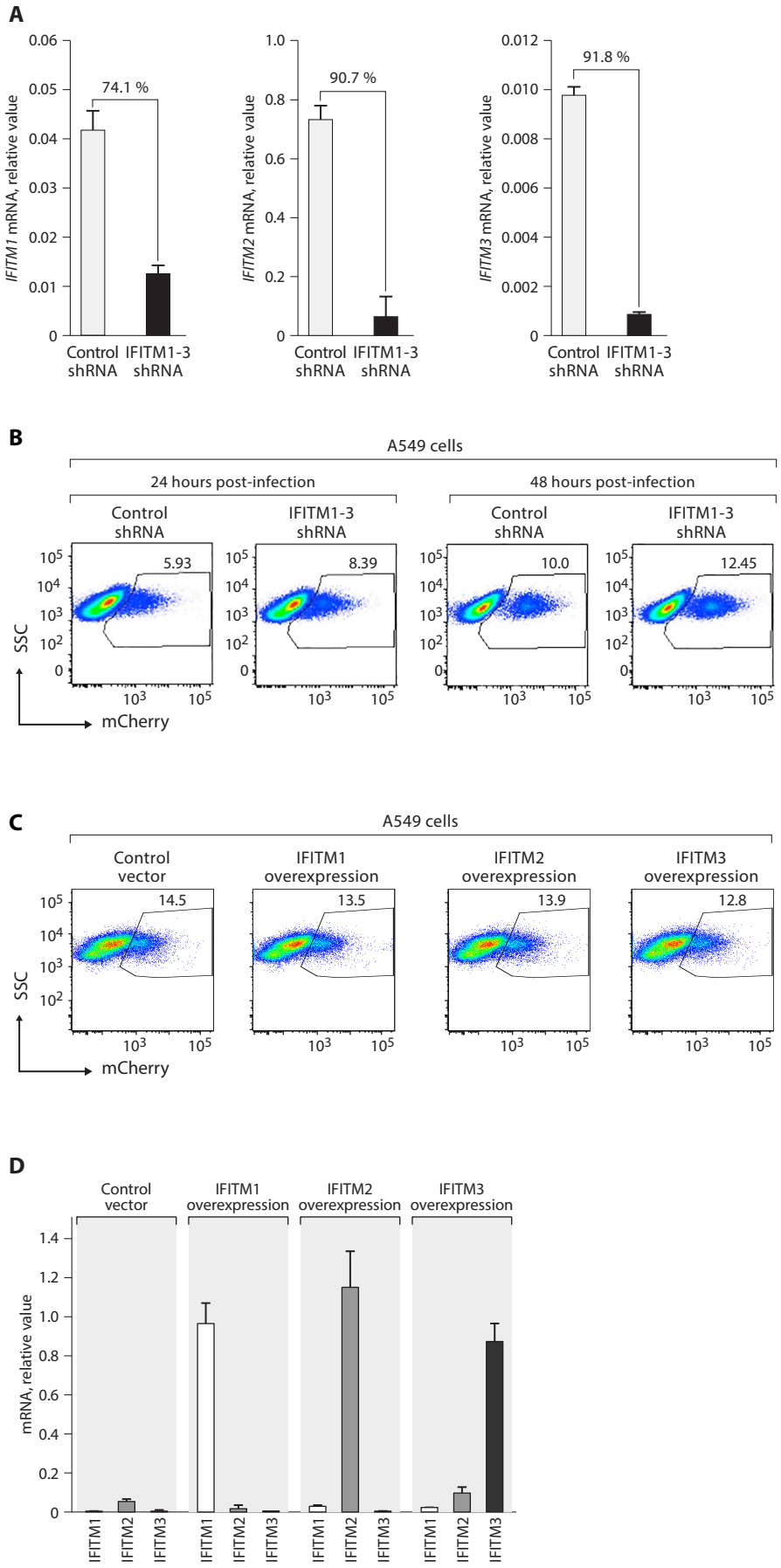

A549 cells
IFITM1
IFITM2
IFITM3
mRNA, relative value
0.6
0.5
0.4
0.3
0.2
0.1
0
0 6 24 48
0 6 24 48
0 6 24 48
Hours post-stimulation

Figure S6


A
IFITM1 mRNA, relative value
74.1 %
Control shRNA
IFITM1-3 shRNA
IFITM2 mRNA, relative value
90.7 %
IFITM3 mRNA, relative value
91.8 %
B
A549 cells
24 hours post-infection
48 hours post-infection
Control shRNA
IFITM1-3 shRNA
5.93
8.39
10.0
12.45
SSC
mCherry
C
A549 cells
Control vector
IFITM1 overexpression
IFITM2 overexpression
IFITM3 overexpression
14.5
13.5
13.9
12.8
SSC
mCherry
D
Control vector
IFITM1 overexpression
IFITM2 overexpression
IFITM3 overexpression
mRNA, relative value
IFITM1
IFITM2
IFITM3

SUPPLEMENTARY FIGURE LEGENDS

Figure S1: RNAi-mediated depletion of IRAK1 or TRAF6 reduces MTb-induced *IFITM1-3* mRNA levels in THP-1 cells. As shown in Figure 1D, knockdown of the adaptor protein MyD88 inhibited *IFITM1-3* transcription in MTb-infected cells. To test whether the downstream signaling molecules IRAK1 and TRAF6 were involved in this inhibition, we transduced THP-1 cells with lentiviral vectors encoding a control shRNA or shRNAs targeting IRAK1 or TRAF6. Cells were infected with MTb strain H37Rv or left uninfected (mock) for 48 hours, and *IFITM1*, *2*, and *3* mRNA levels were measured by qPCR. Results are the mean±SEM from three independent experiments. (*, $p<0.05$; **, $p<0.01$; ***, $p<0.005$).

Figure S2: Pro-inflammatory cytokine induction by TLR2 or TLR4 activation in THP-1 cells. In Figure 1E, we demonstrated that the inflammatory cytokines IL-1β, IL-6, IFNβ, and TNF induced *IFITM1-3* gene expression. To show that TLR2 or TLR4 stimulation resulted in transcriptional induction of these genes in THP-1 cells, we mock-stimulated or stimulated THP-1 cells with the TLR2 agonist Pam3Cys (100 ng/ml) or the TLR4 agonist LPS (100 ng/ml) for 3 or 24 hours and mRNA levels of **A)** *IL1B*, **B)**, *IL6*, **C)** *IFNB1*, and **D)** *TNF*, were measured by qPCR. Results are the mean±SEM from three independent experiments. (*, $p<0.05$; **, $p<0.01$; ***, $p<0.005$).

Figure S3: Detection of increased *IFITM1*, *IFITM2*, or *IFITM3* mRNA levels in THP-1 cells transduced with lentiviral vectors encoding these genes. To validate the specificity of detection of *IFITM1*, *IFITM2*, or *IFITM3* mRNA in Figures 1 and 2, we transduced THP-1 cells with an empty control vector or with individual *IFITM1*, *2*, or *3* expression vectors and, after

stable selection of cells with puromycin, measured mRNA levels by qPCR using primer sets specific for each *IFITM* transcript detailed in the Methods section.

Figure S4: Visualizing MTb attached to the outer membrane of THP-1 cells.
In Figure 3, we analyzed intracellular MTb levels with an Amnis ImageStreamX Mark II machine and IDEAS software by differentiating between the internal region and external region of the cell according to the manufacturer's instructions. Here, we present representative images of extracellular mycobacteria outside of individual cells. From left to right: bright field (gray), MTb-mCherry fluorescence (red), and a merged image of MTb-mCherry fluorescence and bright field. H37Rv-mCherry is visualized in control or IFITM3-overexpressing THP-1 cells (top panel), as well as in THP-1 shRNA control or IFITM-KD cells (bottom panel).

Figure S5: Recombinant IFN-β induction of *IFITM1*, *2*, and *3* mRNA synthesis in A549 cells. In support of studying IFITM3 and MTb localization at endosomal compartments in A549 cells, as is shown in Figure 4, we used IFN-β to stimulate A549 cells and demonstrate that this classical activator of *IFITM* gene expression also induces *IFITM1-3* in this cell line. A549 cells were mock stimulated or stimulated with 20 ng/ml recombinant IFN-β for 6, 24, or 48 hrs and *IFITM1*, *IFITM2*, and *IFITM3* mRNA levels were measured by qPCR using the primers described in the Methods.

Figure S6: IFITM restriction of MTb replication in A549 alveolar epithelial cells. To show that IFITM1-3 also functioned to restrict MTb growth in A549 cells, we performed a similar analysis to that shown in THP-1 cells in Figure 2. These experiments demonstrate that A549 human pulmonary epithelial cells provide an appropriate physiological system for the

examination of the endosomal location of IFITM3 after MTb infection as was performed for Figure 4.

(A) *IFITM1*, *IFITM2*, and *IFITM3* mRNA levels were measured in A549 cells that had been stably transduced with lentiviruses encoding the IFITM1-3 shRNA or the control shRNA.

(B) Flow cytometry analysis of MTb-mCherry infection in control shRNA or IFITM-KD A549 cells at 24 and 48 hrs post-infection.

(C) Flow cytometry analysis of MTb-mCherry levels in control A549 cells or A549 cells overexpressing IFITM1, IFITM2 and IFITM3 at 24 hours post-infection. Results are representative of three independent experiments.

(**D**) Overexpression of IFITM1, IFITM2, or IFITM3 in A549 cells. Enhancement of each *IFITM*'s expression upon lentiviral transduction was confirmed by qPCR using primer sets specific for each *IFITM* transcript.